BIOCHEMISTRY 1

2ND CLASS

UNIVERSITY OF ANBAR
COLLOGE OF SCIENCE
BIOLOGY DEPARTMENT

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Amino Acids

Lecture Two(2)

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References:

Harper's Illustrated Biochemistry

Lippincott Biochemistry

Lehninger Principles of Biochemistry

Stryer Biochemistry

Absorption of ultraviolet light by aromatic amino acids

Tyrosine and tryptophan are significantly more polar than phenylalanine, because of the tyrosine hydroxyl group and the nitrogen of the tryptophan indole ring. Tryptophan and tyrosine, and to a much lesser extent phenylalanine, absorb ultraviolet light (Fig. 1)

This accounts for the characteristic strong absorbance of light by most proteins at a wavelength of 280 nm, a property exploited by researchers in the characterization of proteins.

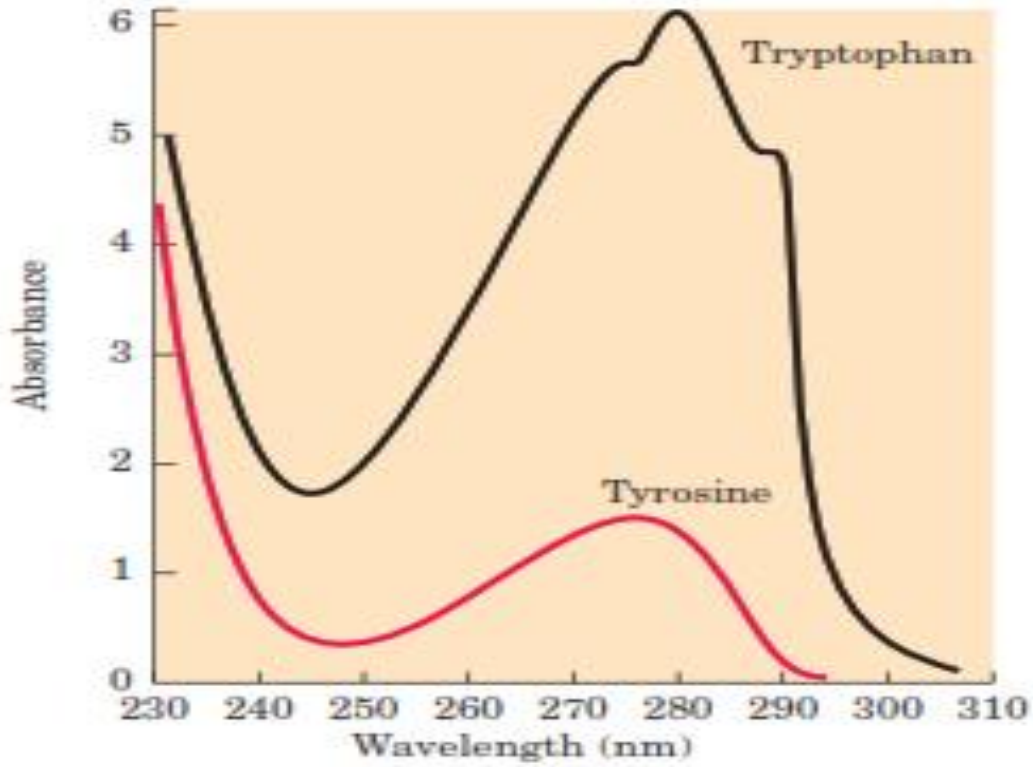


FIGURE 1 Absorption of ultraviolet light by aromatic amino acids. Comparison of the light absorption spectra of the aromatic amino acids tryptophan and tyrosine at pH 6.0. The amino acids are present in equimolar amounts (10^{-3}M) M) under identical conditions. The measured absorbance of tryptophan is as much as four times that of tyrosine. Note that the maximum light absorption for both tryptophan and tyro- sine occurs near a wavelength of 280 nm. Light absorption by the third aromatic amino acid, phenylalanine (not shown), generally con- tributes little to the spectroscopic properties of proteins.

Amino Acids Can Act as Acids and Bases

The amino and carboxyl groups of amino acids, along with the ionizable R groups of some amino acids, func tion as weak acids and bases. When an amino acid lack ing an ionizable R group is dissolved in water at neutral pH, it exists in solution as the dipolar ion, or zwitterion (German for “hybrid ion”), which can act as either an acid or a base (Fig. 2). Substances having this dual (acid-base) nature are amphoteric and are often called ampholytes (from “amphoteric electrolytes”). A simple monoamino monocarboxylic -amino acid, such as alanine, is a diprotic acid when fully protonated; it has two groups, the —COOH group and the —$NH3^{+}$.

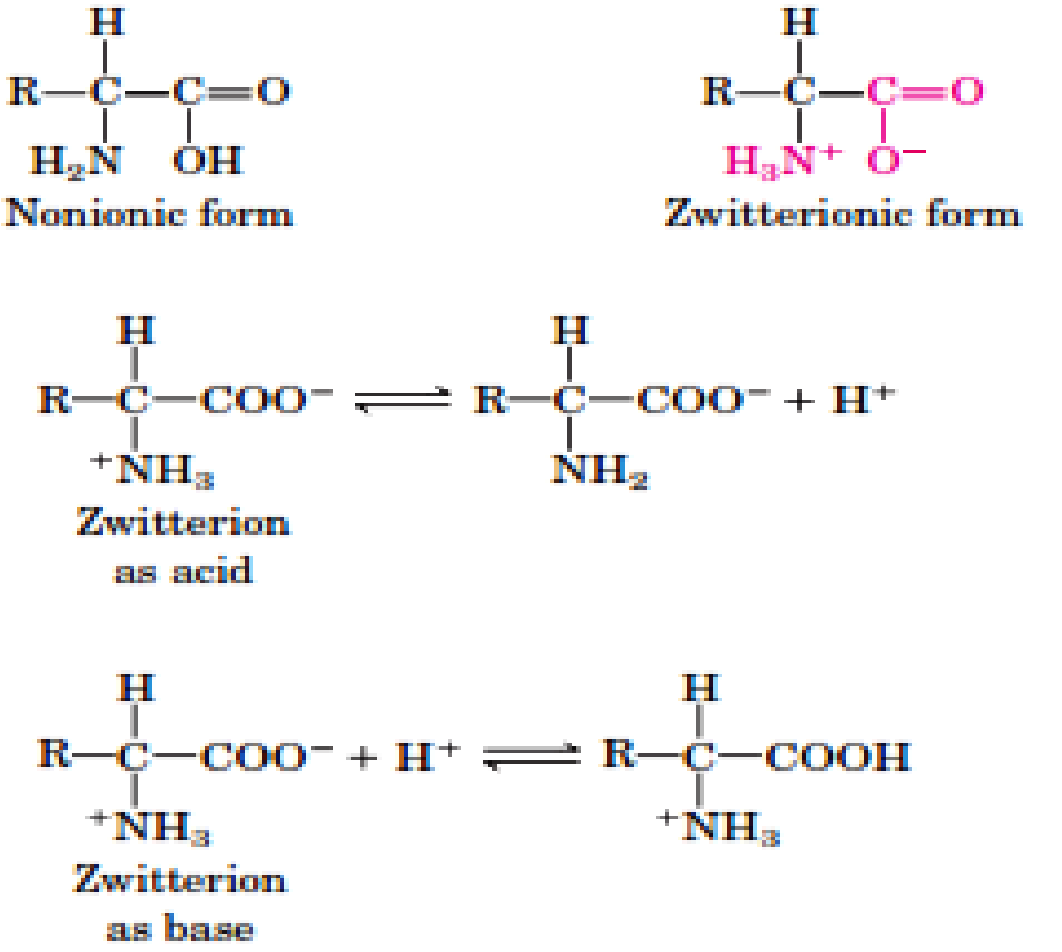
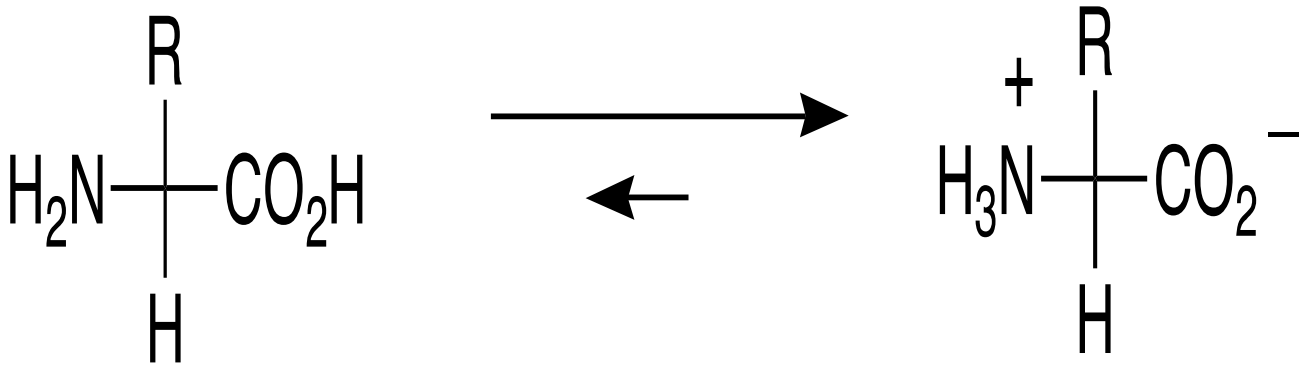
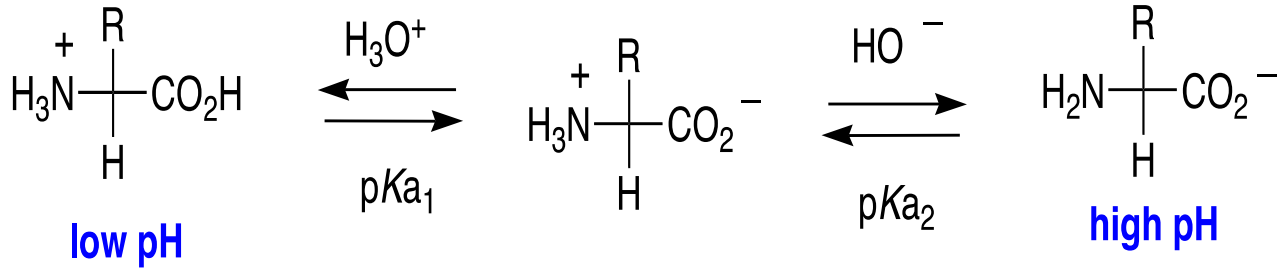


FIGURE 2 Nonionic and zwitterionic forms of amino acids. The nonionic form does not occur in significant amounts in aqueous solu- tions. The zwitterion predominates at neutral pH. A zwitterion can act as either an acid (proton donor) or a base (proton acceptor).

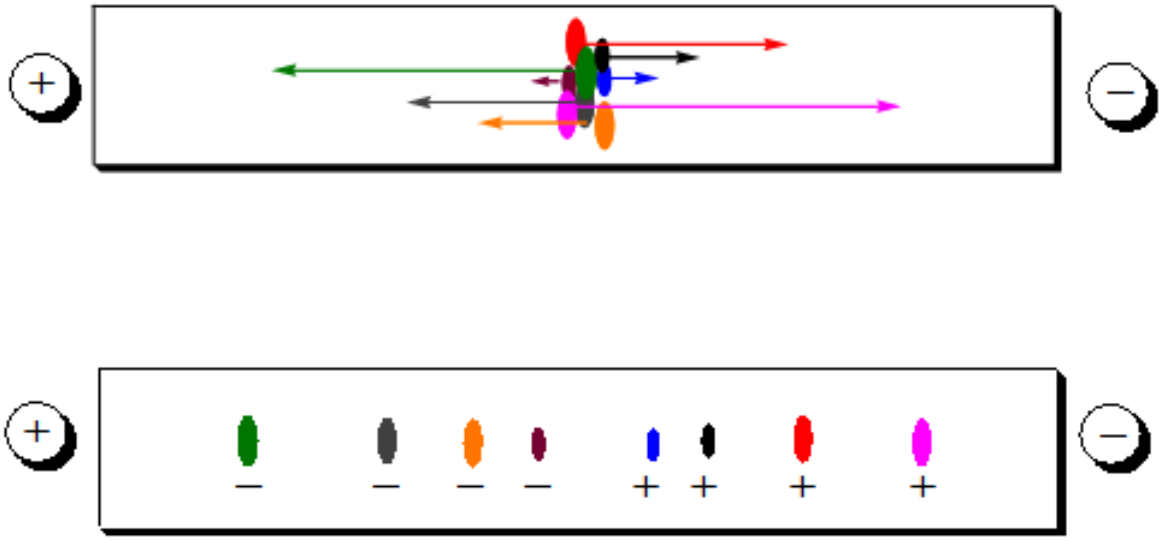
exists largely in a neutral, zwitterionic form (influenced by the nature of the side chain)Amino acids exist as a zwitterion: a dipolar ion having both a formal positive and formal negative charge (overall charge neutral).



Amino acids are *amphoteric*: they can react as either an acid or a base. Ammonium ion acts as an acid, the carboxylate as a base. *Isoelectric point* (pI): The pH at which the amino acid



Electrophoresis: separation of polar compounds based on their mobility through a solid support. The separation is based on charge (pI) or molecular mass.



Titration Curve for Amino Acids

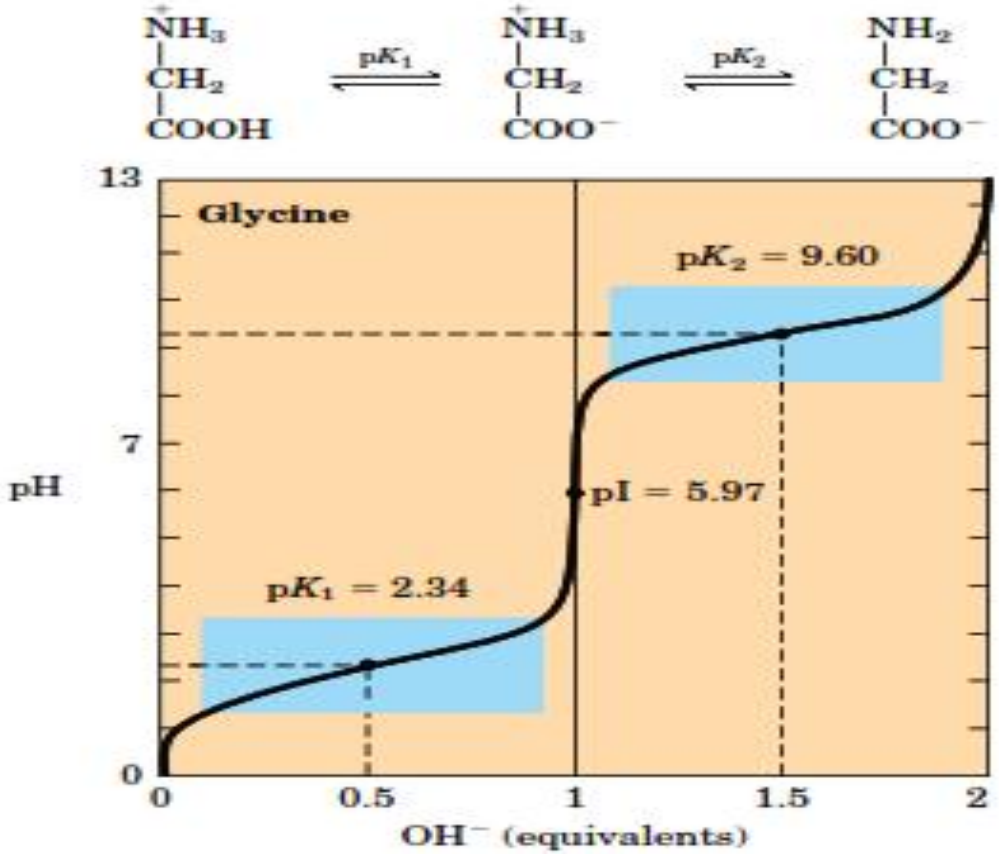
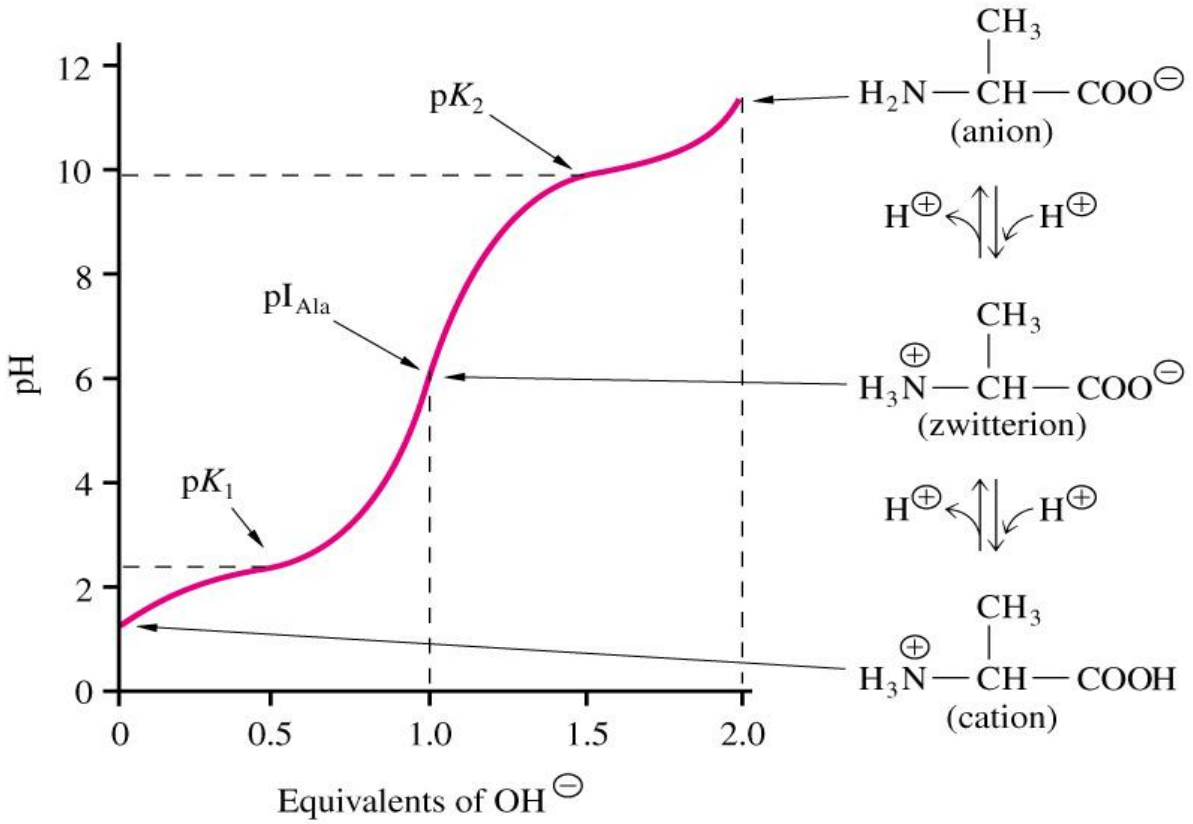


FIGURE 3–10 Titration of an amino acid. Shown here is the titration curve of 0.1 M glycine at 25 °C. The ionic species predominating at key points in the titration are shown above the graph. The shaded boxes, centered at about $pK_1 = 2.34$ and $pK_2 = 9.60$, indicate the regions of greatest buffering power. Note that 1 equivalent of $OH^- = 0.1$ M NaOH added.

Titration Curve for Alanine

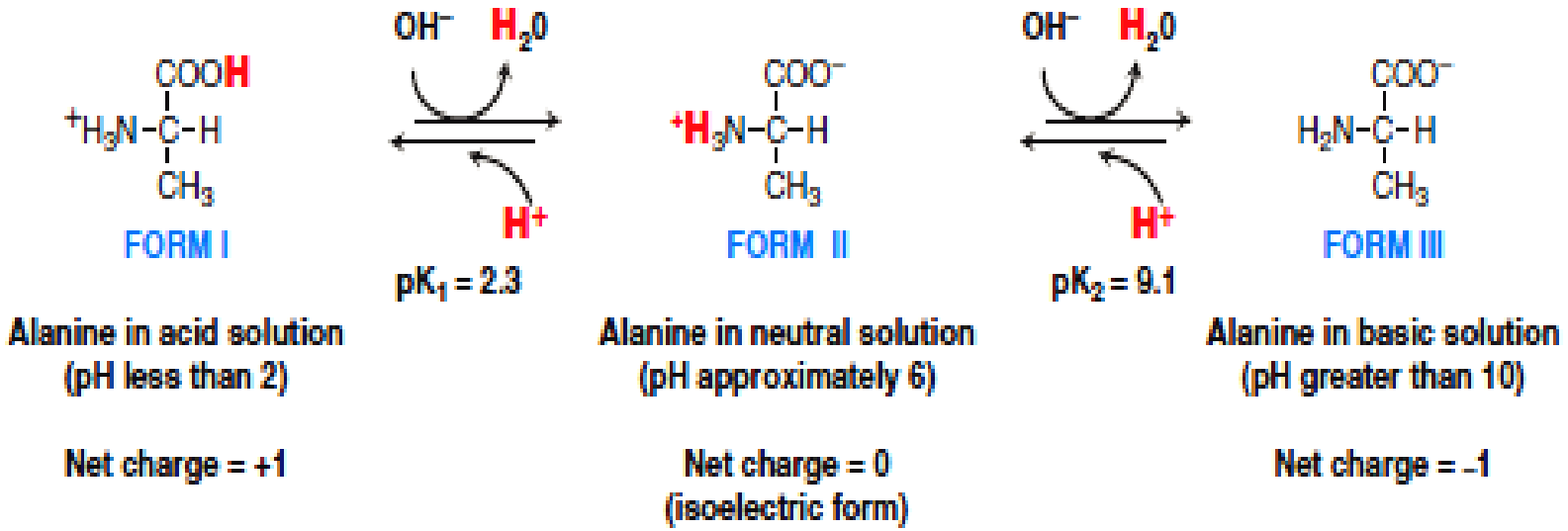


pK_1 carboxylic acid = 2

pK_2 amino group = 10

$pI = (pK_1 + pK_2)/2$

pI (isoelectric point) = the pH at which the number of positive and negative charges on a population of molecules is equal (i.e. no net charge).



Ionic forms of alanine in acidic, neutral, and basic solutions.

Titration Curve for Glutamic Acid

pK_1 carboxylic acid = 2.2

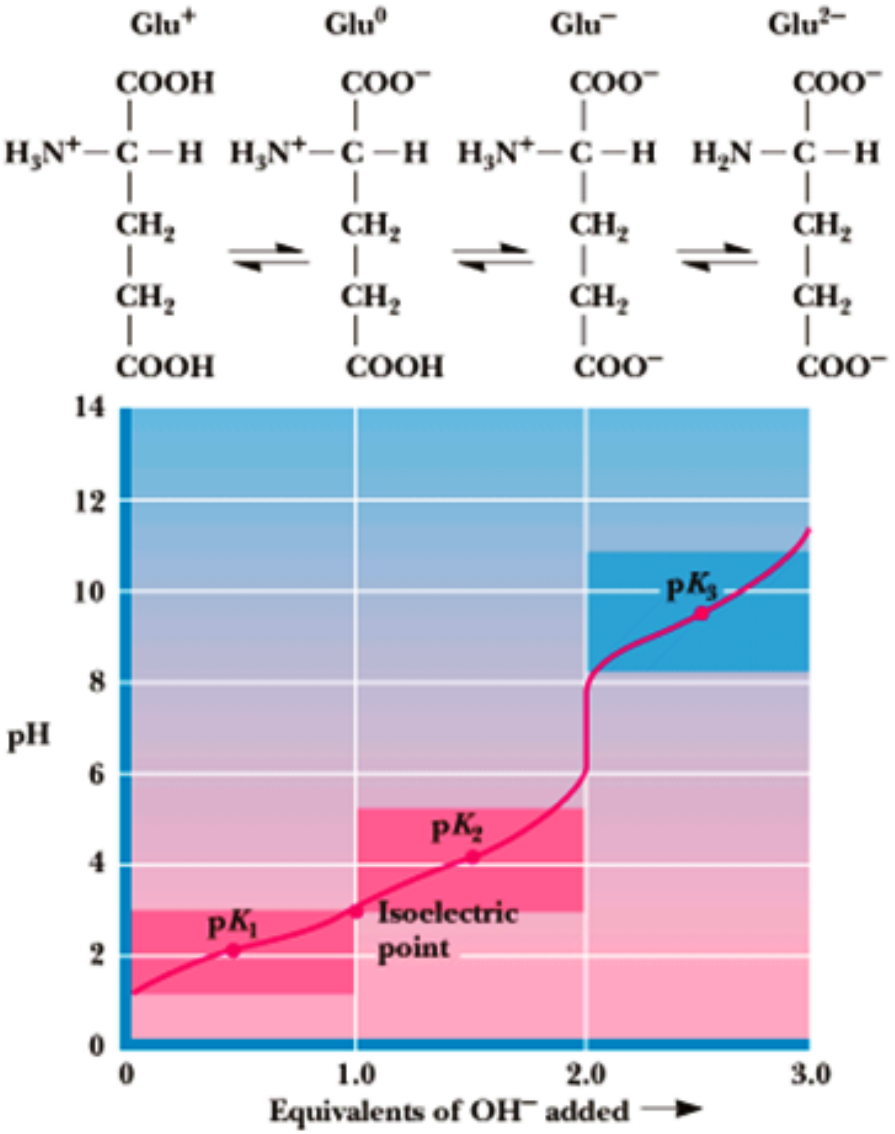
pK_2 R group = 4.3

pK_3 amino group = 9.7

pI = $(pK_1 + pK_2)/2$

pI = (2.2+4.3)/2

pI = 3.25



Titration Curve for Lysine

pK_1 carboxylic acid = 2.2

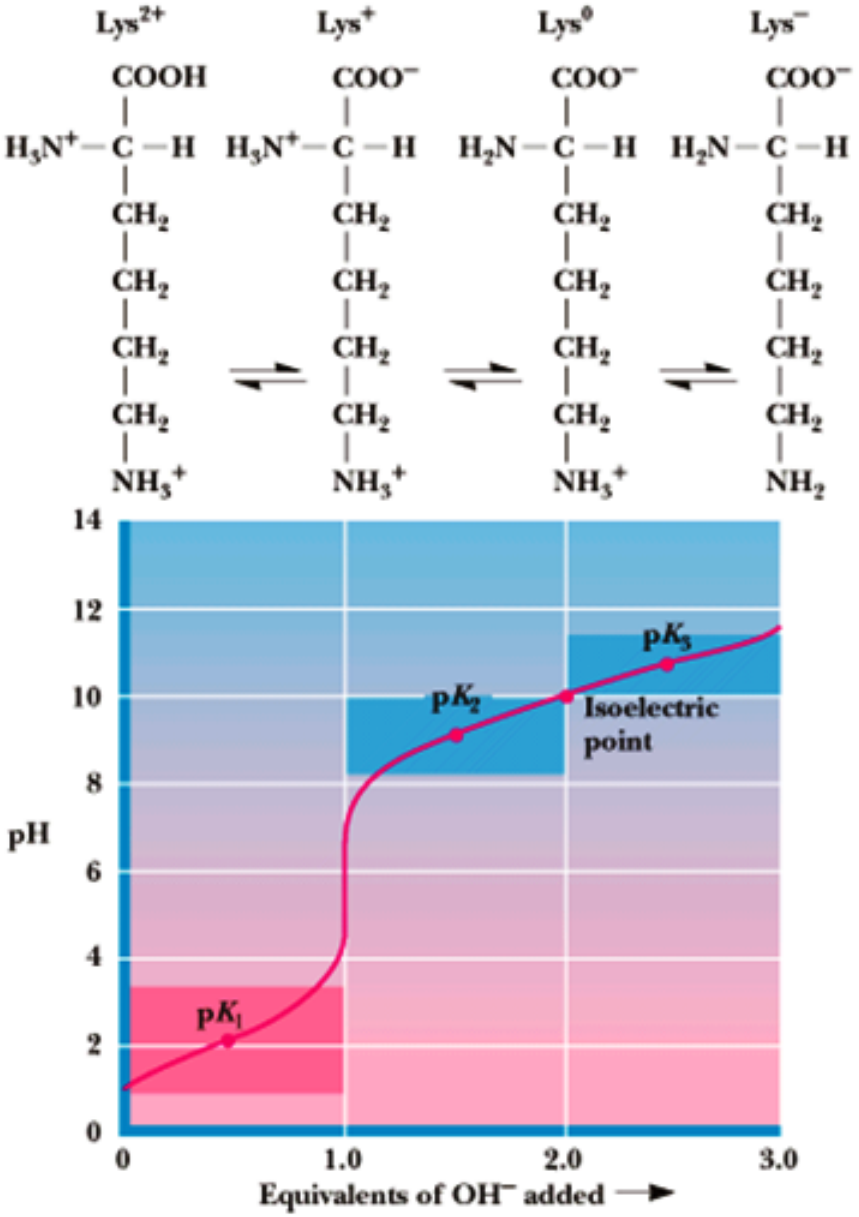
pK_2 amino group = 9.0

pK_3 R group = 10.5

$pI = (pK_2 + pK_3)/2$

$pI = (9+10.5)/2$

$pI = 9.75$



pKa’s of charged amino acids R-groups

- Aspartate/Glutamate = 4.0
- Histidine = 6.0
- Cysteine = 8.4
- Tyrosine = 10.5
- Lysine = 10.5
- Arginine = 12.5

Peptides and Proteins

We now turn to polymers of amino acids, the peptides and proteins. Biologically occurring polypeptides range in size from small to very large, consisting of two or three to thousands of linked amino acid residues. Our focus is on the fundamental chemical properties of these polymers.

Peptides Are Chains of Amino Acids

Two amino acid molecules can be covalently joined through a substituted amide linkage, termed a peptide bond, to yield a dipeptide. Such a linkage is formed by removal of the elements of water (dehydration) from the carboxyl group of one amino acid and the amino group of another (Fig. 3). Peptide bond formation is an example of a condensation reaction, a common class of reactions in living cells. Under standard biochemical conditions, the equilibrium for the reaction shown in Figure 3–13 favors the amino acids over the dipeptide. To make the reaction thermodynamically more favorable, the carboxyl group must be chemically modified or activated so that the hydroxyl group can be more readily eliminated.

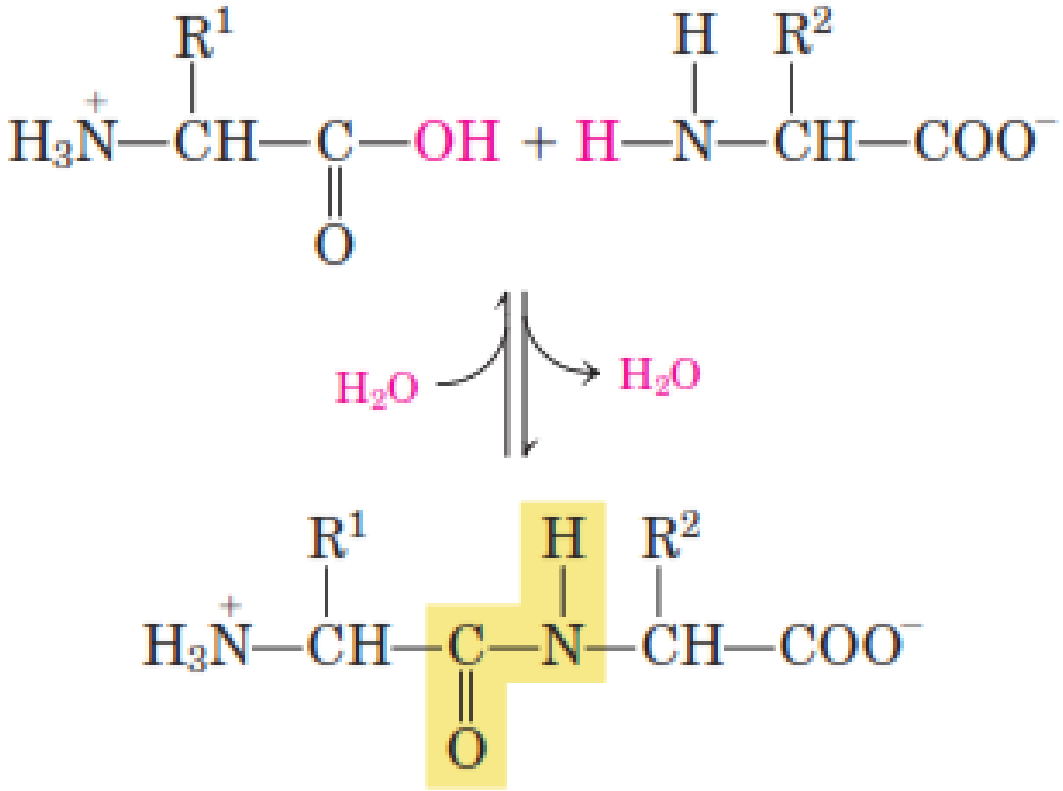


FIGURE 3 Formation of a peptide bond by condensation. The -amino group of one amino acid (with R2 group) acts as a nucleophile to displace the hydroxyl group of another amino acid (with R1 group), forming a peptide bond (shaded in yellow).

Three amino acids can be joined by two peptide bonds to form a tripeptide; similarly, four amino acids can be linked to form tetrapeptides, five to form pentapeptides, and so forth. When a few amino acids are joined in this fashion, the structure is called an oligopeptide. When many amino acids are joined, the product is called a polypeptide. Proteins may have thousands of amino acid residues. Although the terms “protein” and “polypeptide” are sometimes used inter- changeably, molecules referred to as polypeptides generally have molecular weights below 10,000, and those called proteins have higher molecular weights.

Figure 4 shows the structure of a pentapeptide. As already noted, an amino acid unit in a peptide is often called a residue (the part left over after losing the elements of water—a hydrogen atom from its amino group and the hydroxyl moiety from its carboxyl group). In a peptide, the amino acid residue at the end with a free α-amino group is the amino-terminal (or N-terminal) residue; the residue at the other end, which has a free carboxyl group, is the carboxyl-terminal (C-terminal) residue.

When an amino acid sequence of a peptide, polypeptide or protein is displayed, the amino-terminal end is placed on the left, the carboxyl-terminal end on the right. The sequence is read left to right, beginning with the amino-terminal end.

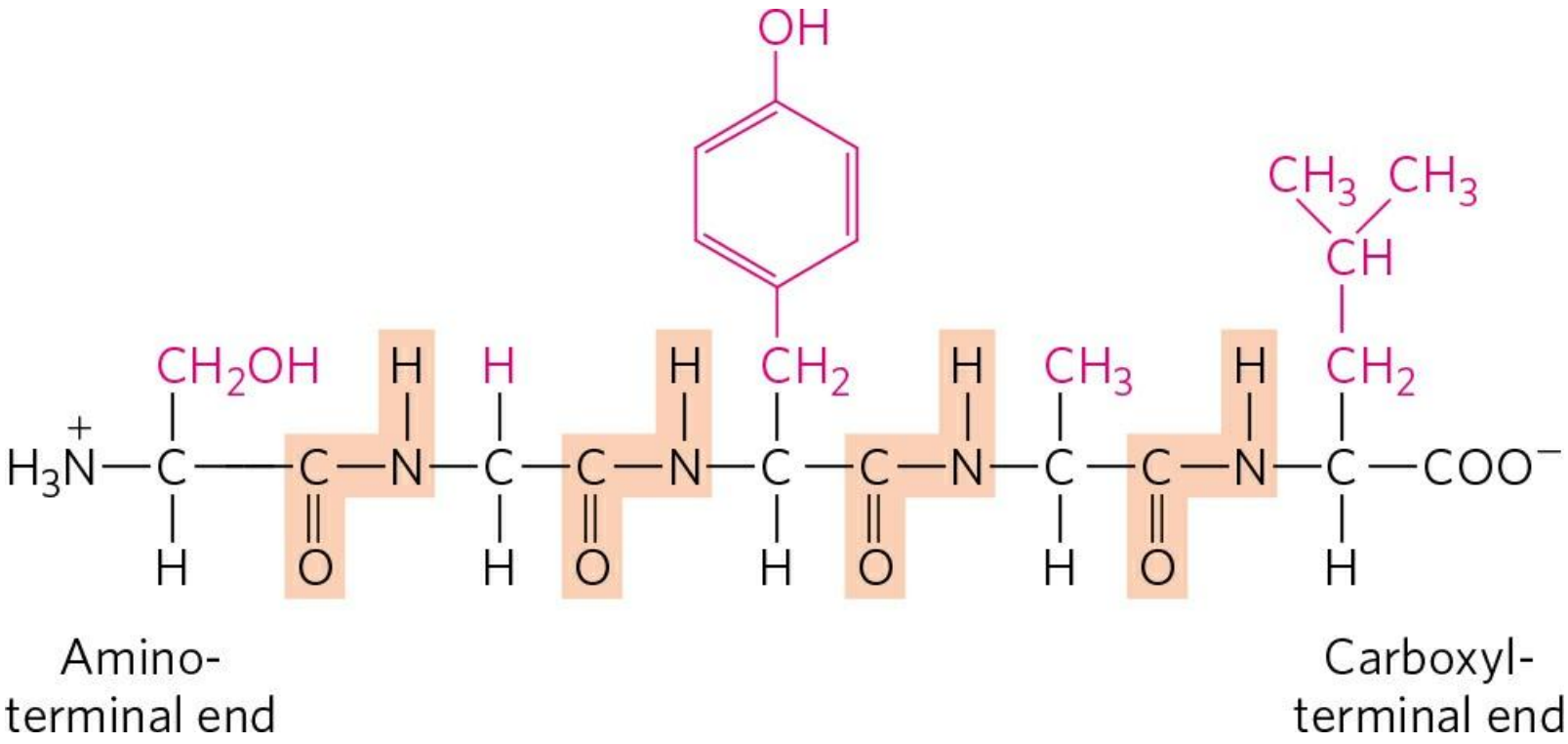
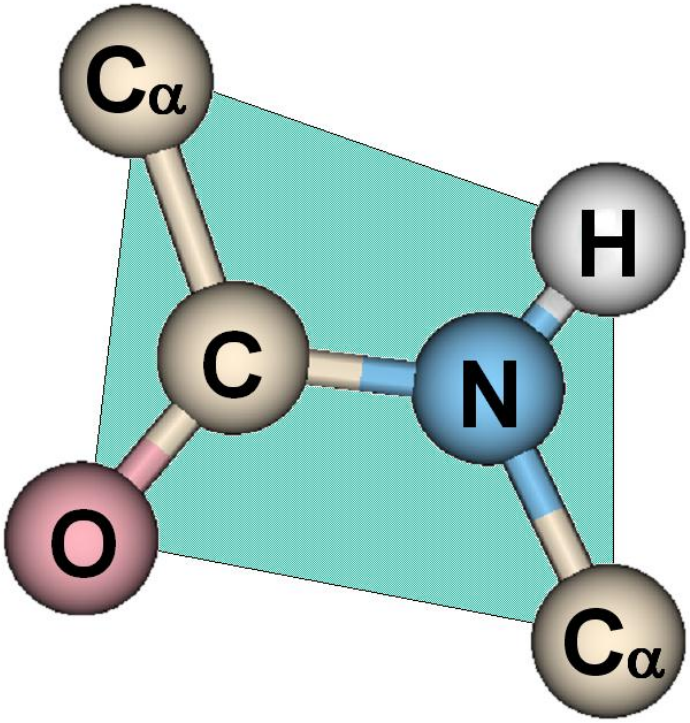
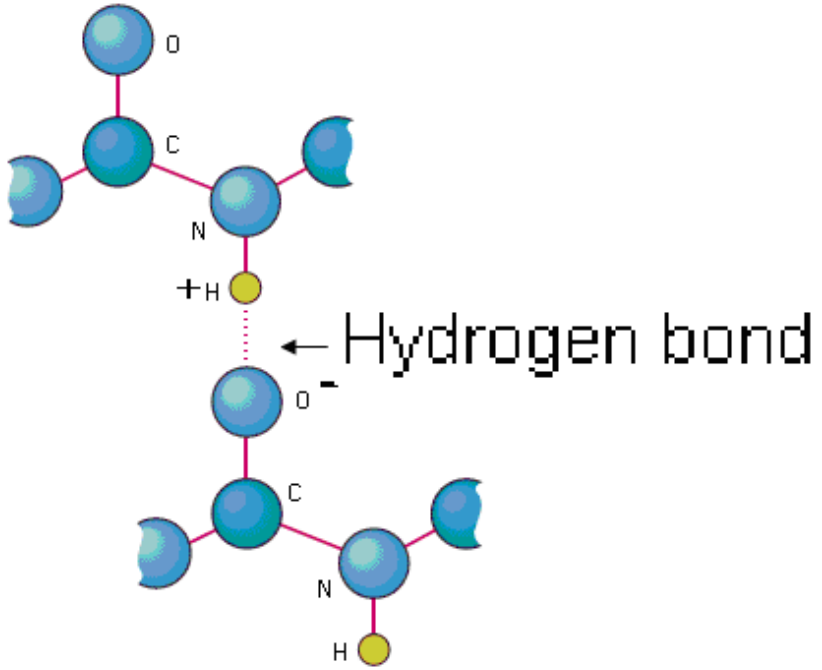


FIGURE 4 The pentapeptide serylglycyltyrosylalanylle ucine , Ser–Gly–Tyr–Ala–Leu, or SGYAL. Peptides are named beginning with the amino-terminal residue, which by convention is placed at the left. The peptide bonds are shaded in light red; the R groups are in red.

Properties of peptide bond:

- The peptide bond is a rigid covalent bond and has **partial double-bond character**.
- The peptide bond is **planar** and **unrotatable**.
- The peptide bond is generally in the **trans conformation**.
- Each peptide bond is able to form maximum **2 hydrogen bonds** with other polar atoms.

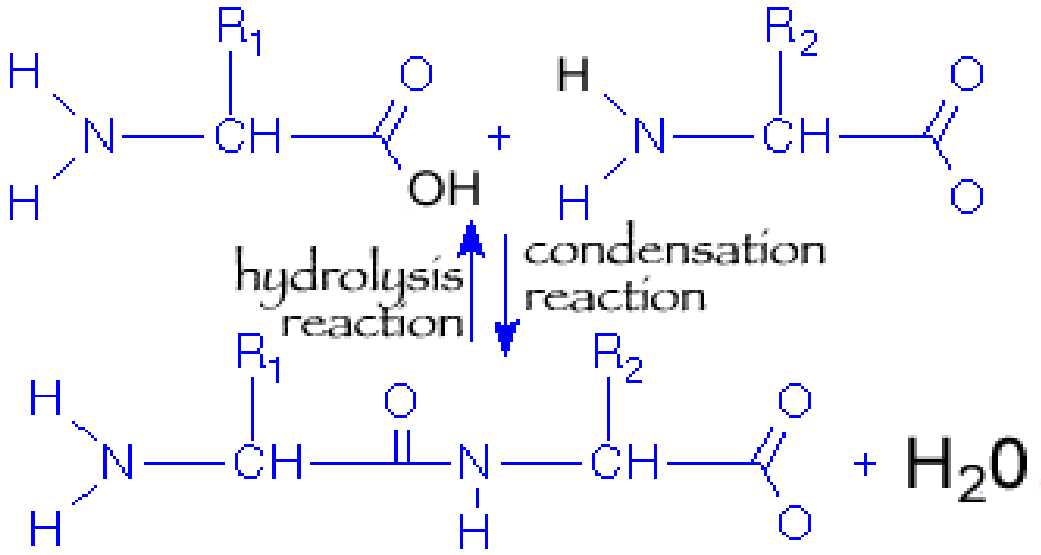




Peptide bond formation is reversible.

A peptide bond can be broken by hydrolysis (the adding of water).

Enzymes such as pepsin and trypsin easily hydrolyze proteins to free amino acids during digestion. However, to break peptide bonds nonenzymically requires harsh conditions (e.g. boiling in 6M HCl for 24 hours).



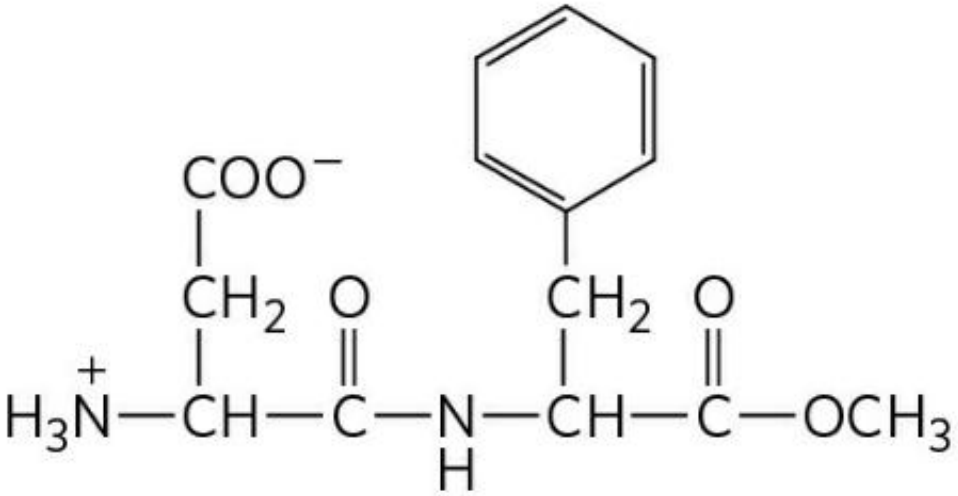
The products of polycondensation of a different number of α-amino acids, bound by peptide bond are named peptides:

- a peptide containing two amino acid units is called a dipeptide;
- one containing three amino acids, a tripeptide;
- one containing a large number, but *less than 50 amino acids*, a peptide;
- one containing 50-100 amino acids – polypeptide;
- if the number of amino acids is *higher then 100*, the polypeptide is called protein.

Biologically Active Peptides and Polypeptides Occur in a Vast Range of Sizes and Compositions

No generalizations can be made about the molecular weights of biologically active peptides and proteins in relation to their functions. Naturally occurring peptides range in length from two to many thousands of amino acid residues. Even the smallest peptides can have biologically important effects. Consider the commercially synthesized dipeptide L-aspartyl-L-phenylalanine methyl ester, the artificial sweetener better known as aspartame or NutraSweet.

Many small peptides exert their effects at very low concentrations. For example, a number of vertebrate hormones are small peptides. These include oxytocin (nine amino acid residues), which is secreted by the posterior pituitary gland and stimulates uterine contractions,



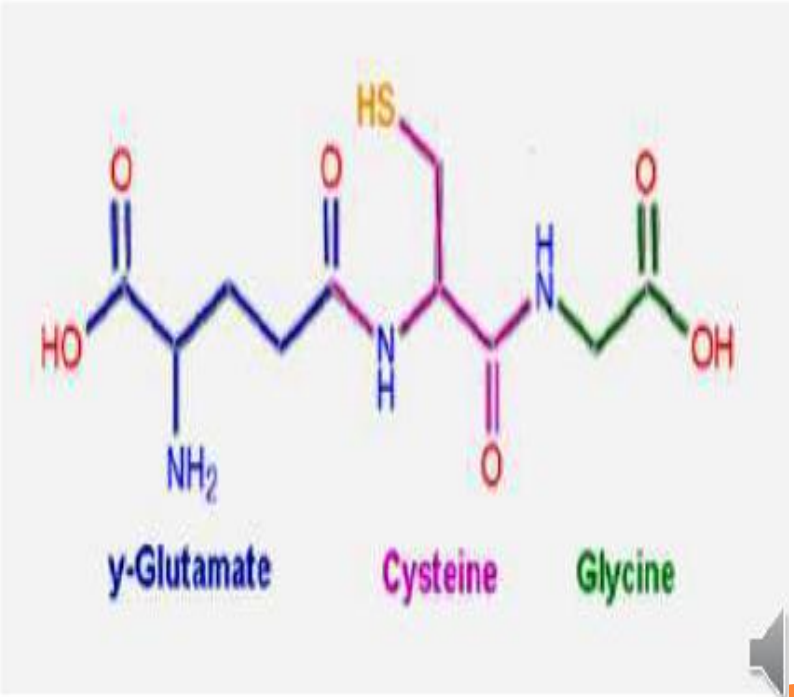
L-Aspartyl-L-phenylalanine methyl ester (aspartame)

and thyrotropin releasing factor (three residues), which is formed in the hypothalamus and stimulates the release of another hormone, thyrotropin, from the anterior pituitary gland. Some extremely toxic mushroom poisons, such as amanitin, are also small peptides, as are many antibiotics.

Biological Activity of small Peptides

- **Glutathione (GSH)** is an antioxidant in plants, animals, fungi, and some bacteria.
- Glutathione is capable of preventing damage to important cellular components caused by Reactive Oxygen Species (ROS) such as free radicals, peroxides, lipid peroxides, and heavy metals

Glutathione: tripeptide (Glutamic acid-Cysteine-Glycine)



Biological Activity of small Peptides

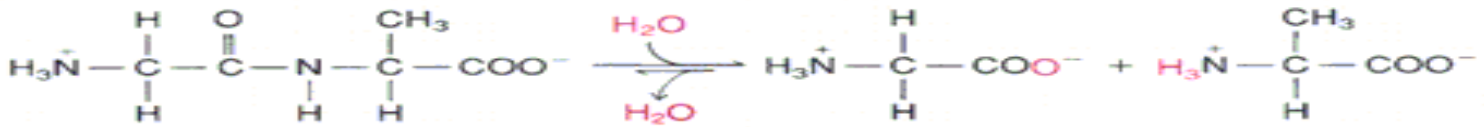
Oxytocin and Vasopressin

- Oxytocin and Vasopressin are a nonapeptide hormones contain 9 amino acids which released by the posterior pituitary.
- Oxytocin is released into the bloodstream as a hormone in labor. Thus, It plays a role in birth (muscular contraction), bonding with the baby, and milk production.
- Vasopressin helps water reabsorption by renal tubules. It called "antidiuretic hormone ADH"

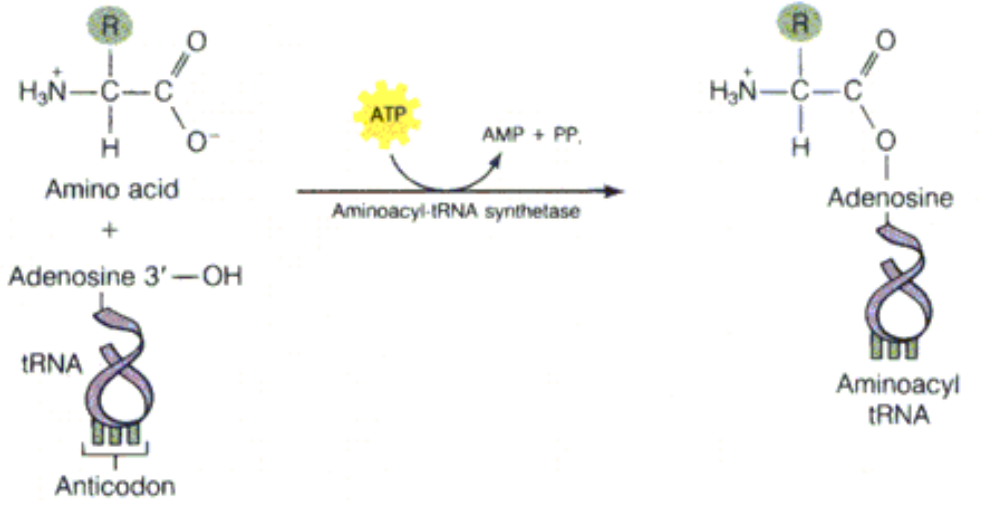
Some proteins consist of a single polypeptide chain, but others, called multisubunit proteins, have two or more polypeptides associated noncovalently. The individual polypeptide chains in a multisubunit protein may be identical or different. If at least two are identical the protein is said to be oligomeric, and the identical units (consisting of one or more polypeptide chains) are referred to as protomers. Hemoglobin, for example, has four polypeptide subunits: two identical α chains and two identical ß chains, all four held together by noncovalent interactions. Each α subunit is paired in an identical way with a ß subunit within the structure of this multisubunit protein, so that hemoglobin can be considered either a tetramer of four polypeptide subunits or a dimer of αß protomers.

We can calculate the approximate number of amino acid residues in a simple protein containing no other chemical constituents by dividing its molecular weight by 110. Although the average molecular weight of the 20 common amino acids is about 138, the smaller amino acids predominate in most proteins. If we take into account the proportions in which the various amino acids occur in an average protein (the averages are determined by surveying the amino acid compositions of more than 1,000 different proteins), the average molecular weight of protein amino acids is nearer to 128. Because a molecule of water (Mr18) is removed to create each peptide bond, the average molecular weight of an amino acid residue in a protein is about 128 − 18 = 110.

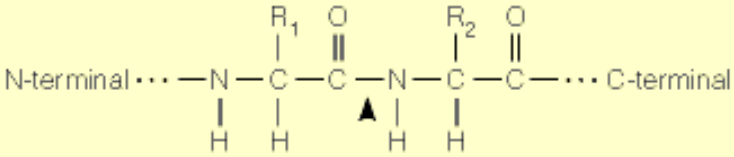
Stability and Formation of the Peptide Bond



- Hydrolysis of peptide bond favored energetically, but uncatalyzed reaction very slow.
- Strong mineral acid, such as 6 M HCl, good catalyst for hydrolysis
- Amino acids must be "activated" by ATP-driven reaction to be incorporated into proteins

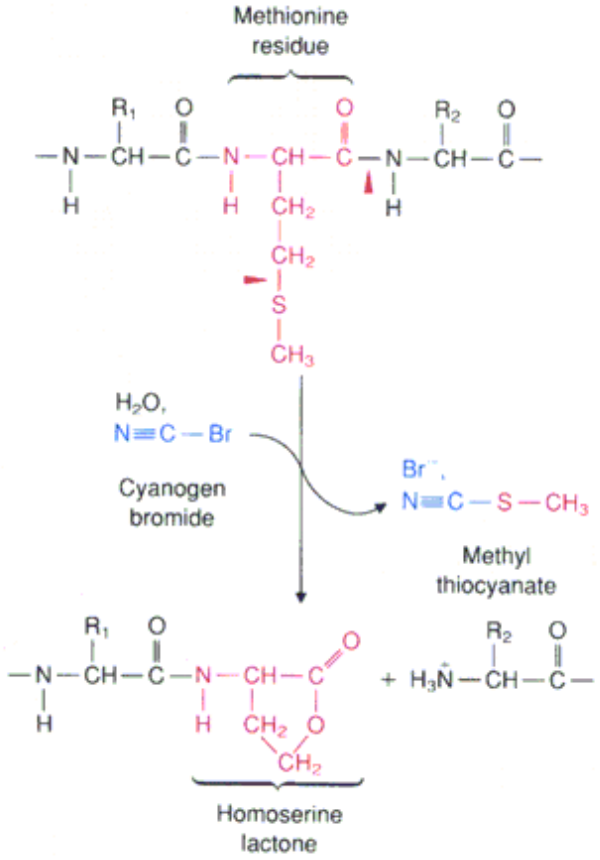


Enzymatic and Chemical Cleavage of Peptide Linkage

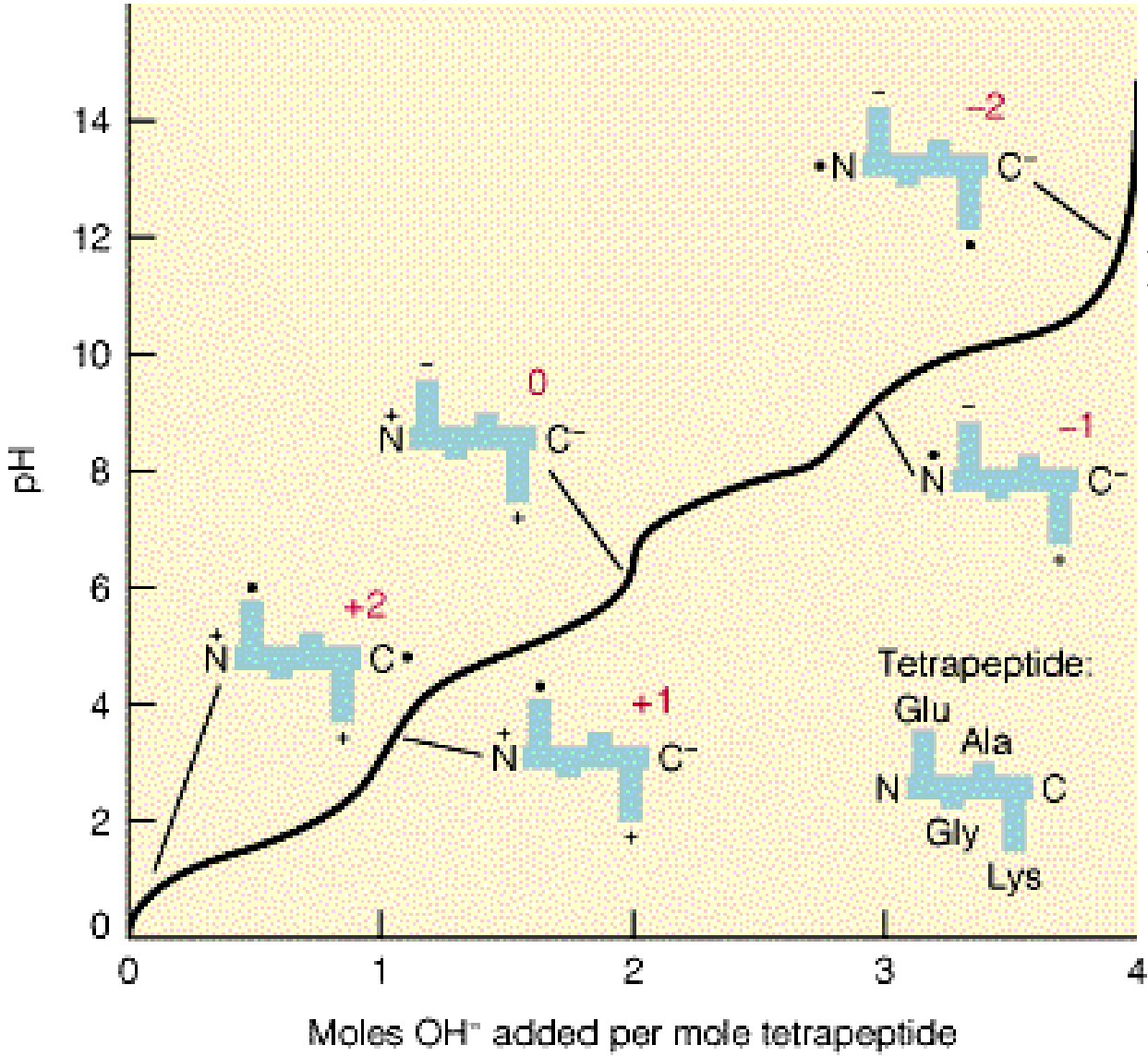


Enzyme	Preferred Site[a]	Source
Trypsin	R_1 = Lys, Arg	From digestive systems of animals, many other sources
Chymotrypsin	R_1 = Tyr, Trp, Phe, Leu	Same as trypsin
Thrombin	R_1 = Arg	From blood; involved in coagulation
V-8 protease	R_1 = Asp, Glu	From *Staphylococcus aureus*
Prolyl endopeptidase	R_1 = Pro	Lamb kidney, other tissues
Subtilisin	Very little specificity	From various bacilli
Carboxypeptidase A	R_2 = C-terminal amino acid	From digestive systems of animals
Thermolysin	R_2 = Leu, Val, Ile, Met	From *Bacillus thermoproteolyticus*

[a]The residues indicated are those next to which cleavage is most likely. Note that in some cases pref erence is determined by the residue on the N-terminal side of the cleaved bond (R_1) and sometime by the residue to the C-terminal side (R_2). Generally, proteases do not cleave where proline is on the other side of the bond. Even prolyl endopeptidase will not cleave if R_2 = Pro.



Titration Curve of a Tetrapeptide



Proteins have pIs

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